1656
#9/Affidavit sent 3/4/02

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

Applicant(s): Glen H. ERIKSON et al.

Serial No: 09/713,177 Group Art Unit: 1656

Filed: November 15, 2000 Examiner: S. Chunduru

Att. Docket No.: E1047/20048 Confirmation No.:

For: TRIPLEX AND QUADRUPLEX CATALYTIC HYBRIDIZATION

SUPPLEMENTAL SUBMISSION

Commissioner for Patents
Washington, DC 20231

Sir:

Supplemental to the Amendment filed January 23, 2002 in response to the Office Action dated August 29, 2001, enclosed please find a complete copy of the Rule 132 Declaration by Dr. Richard A. Collins of the University of Toronto, which is being submitted in the parent application. The copy submitted on January 23, 2002 inadvertently omitted Exhibits B and C.

Should the Examiner believe that anything further is desirable in order to place the application in even better condition for allowance, the Examiner is invited to contact Applicants' undersigned attorney at the telephone number listed below.

Respectfully submitted,
CAESAR, RIVISE, BERNSTEIN,
COHEN & POKOTILOW, LTD.

January 25, 2002

By _____
David M. Tener
Registration No. 37,054
Customer No. 03000
(215) 567-2010
Attorneys for Applicants

Please charge or credit our Account No. 03-0075 as necessary to effect entry and/or ensure consideration of this submission.

CERTIFICATE OF MAILING

I hereby certify that this correspondence re Application No. 09/713,177 is being deposited with the United States Postal services as First Class Mail, postage prepaid, in an envelope addressed to: Commissioner for Patents, Washington, D.C., 20231 on this 25th day of January, 2002.

_____
David M. Tener, Reg. No. 37,054

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

Applicant(s): Glen H. ERIKSON et al.

Serial No: 09/664,827 Group Art Unit: 1655

Filed: September 19, 2000 Examiner: C. Wilder

Att. Docket No.: E1047/20044 Confirmation No.:

For: QUADRUPLEX DNA AND DUPLEX PROBE SYSTEMS

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Washington, DC 20231

Sir:

I, Richard A. Collins, Ph.D., a citizen of Canada, hereby declare and state:

1. The curriculum vitae attached as Exhibit A accurately reflects my professional credentials. As noted in Exhibit A, I am presently employed as a Professor of Medical Genetics at the University of Toronto.

2. Although I have no financial interest in Ingeneus Corp., I previously acted as a paid consultant for Ingeneus Corp. to supervise and report on the blind studies discussed below. I was paid a one-time fee of $1000 by Ingeneus Corp. in the year 2000 for that consultation. I have had no other financial dealings with Ingeneus Corp.

3. The following study was conducted under my direct supervision. The purpose of the study was to provide independent verification of the reality and utility of an assay method developed by Ingeneus Corp. The study was designed to blindly test whether the assay could accurately discriminate wild-type from mutant DNA sequences present in duplex targets by the binding of DNA oligonucleotides.

4. Twelve single-stranded oligodeoxynucleotides (DNAs) were

synthesized by ACGT Corp. (Toronto, Ontario, Canada). The DNAs comprised six complementary pairs of sequences: a wild type and a single base substitution mutant in each of three sequence contexts, designated A, B and C, that differed in G+C content from 33% to 73% (see Exhibit B, attached).

5. DNA concentration was estimated, and six pairs of annealed double-stranded DNA were prepared. After several days of refrigeration, eight aliquots of each of the three wild-type and three mutant annealed samples (48 samples in total) were taken, from which subsets were obtained of eight samples from each of the A, B and C series. These 24 samples were labeled A1 through A8, B1 through B8 and C1 through C8. The identity of these samples was not revealed to the workers conducting the testing. Probe and YOYO were added to each sample, each sample was irradiated with a laser and then the fluorescence of each sample was measured to determine whether it contained wild-type or mutant DNA.

6. The assay correctly identified 23 of the 24 samples tested (96% accuracy) as either wild-type or mutant DNA as noted in my report prepared at that time, attached as Exhibit C.

7. Based on my oversight of the foregoing blind tests, my understanding of the assay and my review of the January 18, 2002 DECLARATION UNDER 37 C.F.R. § 1.132 of Jasmine I. Daksis, I agree with Dr. Daksis' conclusion that the triplex assay is a reality and that her data provide evidence that it does not proceed through a strand invasion mechanism.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine

and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: Jan 21/02

Richard A. Collins, Ph.D.

January 2002

CURRICULUM VITAE

Richard A. Collins

BIOGRAPHICAL INFORMATION

Birthdate: February 28, 1954. Regina, Sask. Canada.
Citizenship: Canadian

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EDUCATION:

B.Sc. University of Regina, Regina, Saskatchewan, Canada, 1975.
Ph.D. University of Regina, Regina, Saskatchewan, Canada, 1979
Thesis title: "Mitochondrial Ribosome Assembly in Wild Type and Mutant Strains of Neurospora crassa". Supervisor: Dr. Helmut Bertrand.

POST-GRADUATE TRAINING:

09/79-12/83 Department of Biochemistry, St. Louis University School of Medicine. Supervisor: Dr. Alan Lambowitz

POSITIONS HELD

07/96-present Professor, Molecular and Medical Genetics, University of Toronto
07/91-06/96 Associate Professor, Molecular and Medical Genetics, University of Toronto
07/89-06/91 Associate Professor, Botany, University of Toronto
01/84-06/89 Assistant Professor, Botany, University of Toronto

FELLOWSHIPS AND AWARDS

01/01-01/07 Canada Research Chair in Molecular Biology
07/96-12/00 Senior Scientist Award, Medical Research Council of Canada
08/92-07/02 Fellow, Canadian Institute for Advanced Research Program in EvolutionaryBiology
07/91-06/96 Scientist Award, Medical Research Council of Canada
09/79-08/82 Post-doctoral fellowship, Medical Research Council of Canada
09/75-08/79 NSERC post-graduate scholarship

RESEARCH INTERESTS:

RNA-catalyzed reactions; RNA structure and folding; mitochondrial gene expression and evolution; intron structure and RNA splicing; molecular evolution.

SCHOLARLY AND PROFESSIONAL WORK
Publications in Refereed Journals (1995 to present):

Sood, V.D. Yekta, S.and Collins, R.A. (2002). The contribution of 2'-hydroxyls to the cleavage activity of the Neurospora VS ribozyme. Nucl. Acids Res. accepted.

Sood, V.D. and Collins, R.A. (2001). Functional equivalence of the uridine turn and the hairpin as building blocks of Neurospora VS ribozyme structure. J. Mol. Biol. 313: 1013-1019.

Hiley, S. and Collins, R.A. (2001). Rapid formation of a solvent-inaccessible core in the *Neurospora* VS ribozyme. EMBO J. 20: 5461-5469.

Andersen, A. and Collins, R.A. (2001). Intramolecular base pair rearrangement by the kissing interaction of the Neurospora VS ribozyme. Proc. Nat. Acad. Sci. 98 7730-7735.

Maguire, J.L. and Collins, R.A. (2001). Effects of cobalt hexammine on folding and self-cleavage of the Neurospora VS ribozyme, J. Mol. Biol. 309: 45-56.

Tillier, E.R.M. and Collins, R.A. (2000). Replication orientation affects the rate and direction of bacterial gene evolution. J. Mol. Evol. 51: 459-463.

Tillier, E.R.M. and Collins, R.A. (2000) Genome rearrangement by replication-directed translocation. Nature Genetics 26: 195-197.

Clarke, G., Collins, R.A., Leavitt, B.R., Andrews, D.F., Hayden, M.R., Lumsden, C.J. and McInnes, R.R. (2000). A one-hit model of cell death in inherited neuronal degenerations. Nature 406: 195-199; Addendum (2001) 409, 542

Tillier, E.R.M. and Collins, R.A.(2000). The contributions of replication orientation, gene direction and signal sequences to base composition asymmetries in bacterial genomes. J. Mol. Evol. 50: 249-257.

Andersen, A. and Collins, R.A. (2000). Rearrangement of a stable RNA secondary structure during VS ribozyme catalysis. Molecular Cell 5: 469-478.

Sood, V.D., Beattie, T.L. and Collins, R.A. (1998). Identification of phosphates involved in metal binding and tertiary folding in the core of the Neurospora VS ribozyme. J. Mol. Biol. 282:741-750.

Olive, J.E. and Collins, R.A. (1998) Spermine switches a Neurospora VS ribozyme from slow cis-cleavage to fast trans-cleavage. Biochemistry 37: 6476-6484.

Rastogi, T. and Collins, R.A. (1998). Smaller, faster VS ribozymes identify the catalytic core of the Neurospora VS ribozyme. J. Mol. Biol. 277: 215-224.

Tillier, E.R.M. and Collins, R.A. (1998). High apparent rate of simultaneous compensatory base pair substitution in ribosomal RNA. Genetics 148: 1993-2002.

Beattie, T.L. and Collins, (1997). Identification of functional domains in the self-cleaving Neurospora VS ribozyme using damage selection. J. Mol. Biol. 267: 830-840.

Rastogi, T.R., Beattie, T.L., Olive, J.E. and Collins, R.A. (1996). A long-range pseudoknot is required for activity of the Neurospora VS ribozyme. EMBO J. 15: 2820-2825.

Olive, J.E., De Abreu, D.M., Rastogi, T., Andersen, A.A., Mittermaier, A.K., Beattie, T.L. and Collins, R.A. (1995). Enhancement of Neurospora VS ribozyme cleavage by tuberactinomycin antibiotics. EMBO J. 14: 3247-3251.

Beattie, T., Olive, J.E. and Collins, R.A. (1995). A secondary structure model for the self-cleaving region of Neurospora VS RNA. Proc. Nat. Acad. Sci. 92, 4686-4690.

Kennell, J.C., Saville, B.J., Mohr, S., Kuiper, M.T.R., Sabourin, J.R. Collins, R.A. and Lambowitz, A.M. (1995). The VS catalytic RNA replicates by reverse transcription as a satellite of a retroplasmid. Genes and Development 9, 294-303.

Guo, H.C.T. and Collins, R.A. (1995). Efficient trans-cleavage of a stem-loop substrate RNA by a ribozyme derived from Neurospora VS RNA. EMBO J. 14: 368-378.

50-mer ssDNAs to be synthesized for INGENEUS RESEARCH by ACGT

Name	Sequence	
CF100	5'-TGG CAC CAT TAA AGA AAA TAT CAT CTT TGG TGT TTC CTA TGA TGA ATA TA-3'	WT (33% GC)
CF101C	3'-ACC GTG GTA ATT TCT TTT ATA GTA GAA ACC ACA AAG GAT ACT ACT TAT AT-5'	
CF102	5'-TGG CAC CAT TAA AGA AAA TAT CGT CTT TGG TGT TTC CTA TGA TGA ATA TA-3'	1 bp G-T (33% GC)
CF103C	3'-ACC GTG GTA ATT TCT TTT ATA GCA GAA ACC ACA AAG GAT ACT ACT TAT AT-5'	
CF104	5'-GAG CAC CAT GAC AGA CAC TGT CAT CTC TGG TGT GTC CTA CGA TGA CTC TG-3'	WT (53% GC)
CF105C	3'-CTC GTG GTA CTG TCT GTG ACA GTA GAG ACC ACA CAG GAT GCT ACT GAG AC-5'	
CF106	5'-GAG CAC CAT GAC AGA CAC TGT CGT CTC TGG TGT GTC CTA CGA TGA CTC TG-3'	1 bp G-T (53% GC)
CF107C	3'-CTC GTG GTA CTG TCT GTG ACA GCA GAG ACC ACA CAG GAT GCT ACT GAG AC-5'	
CF108	5'-GAG CAC CCT CCC AGG CAC GGT CGT CCC TGG TGC GAC CTC CGA CGA GCG TG-3'	WT (73% GC)
CF109C	3'-CTC GTG GGA GGG TCC GTG CCA GCA GGG ACC ACG CTG GAG GCT GCT CGC AC-5'	
CF110	5'-GAG CAC CCT CCC AGG CAC GGT CAT CCC TGG TGC GAC CTC CGA CGA GCG TG-3'	1 bp A-C (73% GC)
CF111C	3'-CTC GTG GGA GGG TCC GTG CCA GTA GGG ACC ACG CTG GAG GCT GCT CGC AC-5'	

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May 16, 2000

To Whom It May Concern:

Re: Summary of blind testing of GeneWeb/Ingeneous fluorescence method for discriminating wild-type from mutant DNA sequences.

Twelve single stranded oligodeoxynucleotides (DNAs)) were synthesized by ACGT Corp (Toronto, Ontario) and delivered directly to my lab at the University of Toronto on May 11, 2000. ACGT is an established supplier of DNAs; their products are widely used by university research labs, including my own lab. The DNAs comprised six complementary pairs of sequences: a wild type and a single base substitution mutant in each of three sequence contexts, designated A, B, and C, that differed in G+C content (individual sequences are attached to this report).

I delivered the DNAs to Dr. Jasmine Daksis at Ingeneous on May 12, and observed her dissolve them, estimate the DNA concentrations, and prepare six pairs of annealed double stranded DNA. I took approximately two thirds of the annealed DNAs, and all of the twelve single stranded DNA solutions to my lab where they were stored refrigerated. The other one third of each sample was left at Ingeneous.

On May 16, 2000 I took the refrigerated samples to Ingeneous where I observed Dr. Daksis dispense eight aliquots of each of the three wild type and three mutant annealed samples (48 samples in total). I retained the remaining material, which I subsequently returned to my lab for storage. The 48 samples were taken to a different room where Dr. Len Zaifman chose a subset of eight samples from each of the A, B and C series (24 samples in total). Dr. Zaifman numbered the samples A1 through A8, B1 through B8 and C1 through C8 and recorded the identity (wild type or mutant) of each on a paper which was sealed into an envelope which he retained. The samples that were not chosen were disposed of such that Dr. Daksis could not know how many wild-type and mutant samples had been chosen from each series. I then observed Dr. Daksis add probe and YOYO, and acquire fluorescence spectra. We discussed her observations, including her predictions about which samples were wild type and which were mutant.

Later on May 16, Mr. Steve Johnston delivered a report containing Dr. Daksis' data and conclusions to my office at the University of Toronto. We were met there by Dr. Zaifman who brought and opened the envelope containing the true identity of each sample and compared this with Dr. Daksis' predictions. In 23 of the 24 cases the predictions were correct.

Yours truly,

Richard A. Collins, Ph.D.
Professor